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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
MEAH, MOHAMMAD Y	
ART UNIT	PAPER NUMBER
1652	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/717,993	LIU ET AL.	
	Examiner	Art Unit	
	MD. YOUNUS MEAH	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 December 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-23,129 and 130* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *11* is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-23 and 129-130 were examined in the previous action.

Claims 1-23 and 129-130 are currently pending in the instant application.

In response to a previous Office action, a non-final action (mailed on 10/21/2008),

Applicants filed a response on 12/17/2008 is acknowledged.

Claims 1-23 and 129-130 are under consideration.

Applicants' arguments filed on 12/17/2008 have been fully considered but they are found unpersuasive. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

35 U.S.C 112 second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 14, 16-17 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 14 is indefinite in the recitation of "first culture medium" as there is no antecedent basis for first culture medium either in claim 1 or claim 13 from which this claim depends.

Claims 16-17 are indefinite in the recitation of "first culture medium" as there is no antecedent basis for first culture medium in claim 1 from which this claim depends.

Claim Rejection - 35 U.S.C 103a

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-7, 12-20, 22-23 and 129-130 are rejected under 35 U.S.C. 103(a) as being obvious over Rajgarhia *et al.* (US pat 7229805) in view of Lee *et al. et al.* (UK patent 2251864, 1995). This rejection is maintained as discussed at length in the previous office action and discussed it again below.

Claims 1 is directed to a method of producing lactic acid, comprising: performing selection on a parent yeast strain that contains an exogenous lactate dehydrogenate gene encoding the amino acid sequence of a lactate dehydrogenate protein of an organism selected from the group consisting *Lactobacillus plantarum*, *bovine*, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, *or Bacillus stearothermophylus* that is capable of being expressed in the parent yeast strain, to yield an acid-tolerant (AT) yeast strain that is capable of growing in a minimal medium at a lower pH than the parent yeast strain; and culturing in a minimal medium the acid-tolerant (AT) yeast strain, wherein the AT yeast strain produces less than about 1 ppm ethanol, wherein the exogenous lactate dehydrogenase gene is capable of being expressed in the AT yeast strain, and wherein a protein resulting from the expression of the exogenous lactate dehydrogenase gene has lactate dehydrogenase activity.

Claims 2-4, 12-23 and 129-130 are directed to the method of claim 1 wherein said AT yeast strain is C_2 carbon source independent grows in minimum cultural

medium having carbohydrate or glucose as carbon source and/or produce lactic acid at pH 3.5 and as low as pH 2.3. Claims 5-7 are directed to the method of claim 1 wherein said AT yeast strain produces 50 g lactic acid/100 g glucose to upto 70 g/100 g glucose.

Rajgarhia et al. teach various recombinant acid tolerant (AT) yeast strains such as *Kluyvermyces,* and *Candida* having deleted pyruvate decarboxylase (PDC, example 15) gene (deletion of PDC gene results no ethanol production, column 4, lines 20-35) expressing, through integration to yeast chromosome or through plasmid, various exogenous LDH genes, including from *Rhizopus oryzae, Bacillus megaterium* (column 28, lines 40-60). Rajgarhia et al. teach that said yeast strain is capable of growing in minimal medium or C_2 independent medium (column 21, lines 20-28) of cell culture at low pH (~2, column 5 lines 25-31, pH 2.5, fig 7). Rajgarhia et al. also teach said strain could produce upto 90gm lactic acid /100gm of glucose (examples 15-16 and table 1) wherein glucose is only carbon source (claim 8, Rajgarhia et al.). Rajgarhia et al. teach variants of recombinant yeast strains expressing exogenous LDH genes with the highest specific productivity during the anaerobic phase can be found not only to produce lactic acid faster, but also achieve a higher concentration at a lower pH, than the variants with lower specific productivity (example 15, column 38, lines 30-35) However Rajgarhia et al. do not teach a method of selection of most acid tolerant, AT, yeast strain from the parent yeast strain expressing exogenous LDH gene.

It is well known in the art that production of lactic acids in a cultural medium drop the pH of the medium (Lee et al. page 3) and most of the lactic acid producing bacteria

do not grow at lower pH (Lee et al. page 1). Yeast cells on the other hand are viable at low pH (Rajgarhia et al. column 1 lines 25-46). Lactic acid is an industrially important chemical and to increase the yield of lactic acid, a microbial environment that can tolerate low pH is desired. Selection of microbial cell that grow at most low pH is advantageous for increased production of lactic acid. Rajgarhia et al. teach variants of recombinant yeast strains expressing exogenous LDH genes with the highest specific productivity during the anaerobic phase can be found not only to produce lactic acid faster, but also achieve a higher concentration at a lower pH, than the variants with lower specific productivity (example 15, column 38, lines 30-35).

Lee *et al.* teach the method of selection of mutant *lactobacillus* cell which is viable at low pH and produce lactic acid at low pH, wherein parent *lactobacillus* cell is cultured at various low pH and selection is made for the mutant *lactobacillus* cell that viable at lower pH than the parent strain (pages 6-7 and claim 9 of Lee et al.).

Therefore, one of ordinary skill in prior art would have been **motivated** to use Rajgarhia *et al.* yeast strains expressing exogenous LDH genes which show the highest specific productivity during the anaerobic phase, produce lactic acid faster and higher concentration at a lower pH (example 15, column 38, lines 30-35), grow it at various lower pH and minimal medium using methods as described in examples 8, 15 (column 35, lines 35-53 and column 38, lines 36-44) and select the viable yeast cells that produce lactic acid at the lowest pH using the selection procedure of Lee et al. One of ordinary skill in the art would have been motivated to do so because recombinant acid tolerant yeast strains expressing exogenous LDH genes is used for the production of

lactic acid, an industrially useful chemical. One of ordinary skill in the art would have been also motivated to select the most acid tolerable yeast strain that produce the highest amount of lactic acid by growing acid tolerant yeast strain and lowering pH and selecting the most viable strain at the lowest pH, because i) use of acid tolerant yeast strains expressing exogenous LDH gene for the efficient production of lactic acid is well known in the art, ii) it is easy to purify lactic acid from yeast media at low pH, iii) yeast strain that produce lactic acid at lowest pH will produce most lactic acid and require least purification step (column 25, lines 20-50 of Rajgarhia et al.).

As such it would have been obvious to one of ordinary skill in the art use Rajgarhia et al's yeast *Kluyvermyces* strains expressing (through integration to yeast chromosome or through plasmid) *Rhizopus oryzae* LDH gene and grow it at various lower pH and minimal medium using methods as described in examples 8, 15 (column 35, lines 35-53 and column 38, lines 36-44) and then make a selection of most acid tolerant (AT) viable strain using the selection procedure of Lee et al. The expectation of success is high, because the above cited references define the status of the prior art in the successful method of obtaining most acid tolerant yeast strain for the production of lactic acid faster and higher concentration at a lower pH.

Arguments and response

Applicants' argue, at pages 7-10 of their amendment of 12/ 17/08 that Rajgarhia *et al.* do not teach acid tolerant (AT) yeast strain because Rajgarhia et al yeast strain does not produce lactic acid at lower pH than that of parent yeast strain; 2) by combining Lee et al teaching with Rajgarhia et al's, one of ordinary skill in the art would not have

reasonable expectation of success at arriving at the applicants invention. Applicant further argue that Lee et al teach selection of Lactobacillus by culturing Lactobacillus in milk media, does not teach culturing yeast strain expressing exogenous LDH gene and therefore one of ordinary skill in the art would not expect to select yeast strain growing in milk medium. Applicant further argue that Lee et al do not teach lowering pH of the culturing medium below 6.2 and Lee's selection system yield Lactobacillus strain viable at pH 3.4, therefore; applicants conclude that selection of acid tolerant yeast strain capable of producing lactic acid at pH values 3-4 or at about 2.8 is beyond expectation of the person of ordinary skill in the art.

Applicants' arguments filed on 12/17/08 have been fully considered, but they are found unpersuasive. Applicants argument that Rajgaria et al do not teach acid tolerant (AT) yeast strain because Rajgarhia et al yeast strain does not produce lactic acid at lower pH than that of parent yeast strain is not convincing because Rajgarhia et al. teach variants of recombinant yeast strains expressing exogenous LDH genes with the highest specific productivity during the anaerobic phase can be found not only to produce lactic acid faster, but also achieve a higher concentration at a lower pH, than the variants with lower specific productivity (example 15, column 38, lines 30-35). Therefore Rajgarhia et al teach Acid tolerant variants yeast strain (that produce lactic acid at pH about 2.3-2.5). Regarding argument of growing yeast strain in milk medium of Lee et al, as explained above, the teaching of Lee et al is used for its teaching of selection procedure of acid tolerant strain (the selection procedure is universal, and it can be applied to any microorganism strain). Therefore cultural media and pH used in

the method of production of yeast strain is that of Rajgarhia et al, not Lee et al. Rajgaria et al teach growing yeast strain at different pHs (as low as pH 2.5-2.8, fig 7, fig 1) and medium (example 8, and 15) as discussed above). One of ordinary skill in art can use the selection procedure of Lee et al and apply to the yeast strain production method of Rajgaria et al (growing yeast at a cultural comprising minimal medium and various pHs, as low as about 2.5 and below, example 8 and 15) and select a yeast strain which is viable at most lower pH (Rajgarhia et al yeast strain viable and produce lactic acid at a pH about 2.5 (Fig 7, before selection).

Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rajgarhia *et al.* (US pat 7229805) in view of Lee *et al.* (UK patent 2251864, 1995) as applied to claims 1-7, 12-20, 22-23 and 129-130 above, and further in view of House *et al.* (US2003/0228671). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claim 8 is directed to the method of claim 1 wherein said AT yeast strain produce less than 1 ppm of glycerol.

Rajgarhia *et al.* teach various recombinant acid tolerant (AT) yeast strains, i.e., *Kluyvermyces, Candida, etc*, having deleted pyruvate decarboxylase (PDC, example 15) gene (deletion of PDC gene results no ethanol production, column 4 lines 25-34 of Rajgarhia et al.) expressing, through integration to yeast chromosome or through plasmid, various exogenous LDH genes, including from *Rhizopus oryzae* (column 28, lines 41-61). Lee *et al.* are described above.

However Rajgarhia et al. do not teach AT yeast strain producing less than 1 ppm of glycerol.

House *et al.* (US2003/0228671) teach method of producing lactic acid using recombinant acid tolerant yeast strains expressing exogenous LDH gene without producing any ethanol or glycerol (page 17, pargh 0209).

As such it would have been obvious to one of ordinary skill in the art to use House *et al.* (US2003/0228671) recombinant acid tolerant yeast strains expressing exogenous LDH gene grow it different media and pHs as taught by Rajgarhia et al. (US pat 7229805) and make a selection of most AT yeast strain using the selection procedure of Lee et al and use it for the efficient production of lactic acid without producing any ethanol or glycerol.

Arguments and response

Applicants' argument, at page 8 of their amendment of 12/17/08, against claim 8 have been fully considered, but they found unpersuasive, as explained above in the response against the argument for claims 1-7, 12-20, 22-23 and 129-130.

Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rajgarhia *et al.* (US pat 7229805) in view of Lee *et al.* (UK patent 2251864, 1995) as applied to claims 1-7, 12-20, 22-23 and 129-130 above, and further in view of Rajgarhia *et al.* (US2004/0029256). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claim 21 is directed to the method of claim 1 wherein said AT yeast strain expresses exogenous LDH gene from *lactobacillus plantarum.*

Rajgarhia *et al.* (US pat 7229805) teach various recombinant acid tolerant (AT) yeast strains such as *Kluyvermyces* and *Candida* having deleted pyruvate decarboxylase (PDC, example 15) gene (deletion of PDC gene results no ethanol production (column 4 lines 25-34) expressing, through integration to yeast chromosome or through plasmid, various exogenous LDH genes, including from *Rhizopus oryzae* (column 28, lines 41-61). Lee *et al.* are described above.

However Rajgarhia *et al.* (Pat 7229805) do not teach AT yeast strain expressing exogenous LDH gene from *lactobacillus plantarum*.

Rajgarhia *et al.* (US2004/0029256) teach recombinant acid tolerant yeast strains expressing exogenous LDH gene from *lactobacillus plantarum* (claim 10 of Rajgarhia et al US2004/0029256).

As such, it would have been obvious to one of ordinary skill in the art to use Rajgarhia *et al.* (US2004/0029256) recombinant acid tolerant yeast strains expressing exogenous *lactobacillus plantarum* LDH gene grow it different media and pHs as taught by Rajgarhia et al. (US pat 7229805) and make a selection of most AT yeast strain using the selection procedure of Lee et al and use it for the efficient production of lactic acid.

Arguments and response

Applicants' argument, at page 11 of their amendment of 12/ 22/08, against claim 11 have been fully considered, but they found unpersuasive, as explained above in the response against the argument for the claims 1-7, 12-20, 22-23 and 129-130.

Claims 9-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rajgarhia et al. (US Pat 7229805) in view of Lee et al. (UK patent 2251864, 1995) as applied to claims 1-7, 12-20, 22-23 and 129-130 above, and further in view of Porro et al (US 7049108). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claims 9-10 are directed to the method of claim 1 wherein said AT yeast strain *comprise Saccharomyces or Saccharomyces cerevisiae.*

Rajgarhia *et al.* (US Pat 7229805) teach various recombinant acid tolerant (AT) yeast strains, i.e., *Kluyvermyces, Candida, etc*, having deleted pyruvate decarboxylase (PDC, example 15) gene (deletion of PDC gene results no ethanol production, (column 4 lines 24-35) expressing, through integration to yeast chromosome or through plasmid, various exogenous LDH genes, including from *Bacillus megaterium* (column 28, lines 41-61). Lee et al. are described above.

However Rajgarhia *et al.* (Pat 7229805) do not teach AT yeast strain comprising *Saccharomyces or Saccharomyces cerevisiae.*

Porro et al teach recombinant *Saccharomyces cerevisiae* yeast strain expressing various exogenous LDH genes including from *Bacillus megaterium,* wherein said yeast strain comprise deleted PDC genes so that it produce no ethanol.

However Porro et al. do not teach a method of selection of AT yeast strain from the parent yeast strain expressing exogenous LDH gene.

As such, it would have been obvious to one of ordinary skill in the art to use Porro et al recombinant acid tolerant *Saccharomyces cerevisiae* yeast strain expressing exogenous LDH gene from *Bacillus megaterium* grow it different media and pHs as taught by Rajgarhia et al. (US pat 7229805) and make a selection of most AT yeast strain using the selection procedure of Lee et al and use it for the efficient production of lactic acid.

Arguments and response

Applicants' argument, at pages 11-12 of their amendment of 12/17/08, against claim 8 and 9 have been fully considered, but they found unpersuasive, as explained above in the response against the argument for claim 1-7, 12-20, 22-23 and 129-130.

Conclusion

Claim 11 is allowable.

Claims 1-10, 12-23 and 129-130 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mohammad Meah whose telephone number is 571-272-1261. The examiner can normally be reached on 8:30-5PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, NASHAAT T NASHED can be reached on 571-272-0934. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For

more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Mohammad Younus Meah
Examiner, Art Unit 1652

/Nashaat T. Nashed/
Supervisory Patent Examiner, Art Unit 1652